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THE EFFECT OF pH ON SUGAR TRANSPORT AND ION DISTRIBUTION IN KIDNEY CORTEX CELLS

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SUMMARY

The effect of pH (range: 6.2–8.2) on active sugar transport and the steady-state ionic distribution was studied using slices of rabbit kidney cortex. Sugars used: 2-deoxy-D-glucose, 2-deoxy-D-galactose, D-galactose and α-methyl-D-glucoside.

1. An increase of pH from 6.2 to 8.2 affected sugar transport as follows: (a) A 3-fold increase in the rate and steady-state accumulation of 2-deoxy-D-glucose both in Na^+- and Na^+-free (Li^+) salines was found; changes of pH affected the K_m of 2-deoxy-D-glucose transport. (b) A marked decrease of the accumulation of 2-deoxy-D-galactose in both Na^+- and Li^+-salines was observed, the transport K_m being affected. (c) A 4-fold stimulation of D-galactose transport in Na^+-saline took place, with only a minimal effect on the Na^+-independent transport. This pH effect was reversible. An increase of pH from 7.2 to 8.2 markedly decreased the efflux of D-galactose from the cells. Evidence for complex influx kinetics was obtained. (d) No major changes of the steady-state accumulation of α-methyl-D-glucoside in Na^+-salines could be detected; in Li^+-saline, an accumulation of α-methyl-D-glucoside at pH 6.2 was found ($[S]_i/[S]_o$: 1.725 ± 0.101 (S.E.). This active accumulation at $[Na^+]_o = 0$ (and $[Na^+]_i < 3$ mM) was inhibited to $[S]_i/[S]_o$ values below 1.0 by anaerobiosis, and by 0.1 mM dinitrophenol or phlorrhizin, but was insensitive to 0.5 mM ouabain or acetazolamide.

2. The increase of pH from 6.2 to 8.2 affected as follows the steady-state tissue water and the respective electrochemical ionic potentials (referred to $E_{^{36}Cl^-}$): (a) In Na^+-salines, tissue water, $E_{^{36}Cl^-}$ and E_{K^+} were minimally altered; E_{Na^+} and E_{H^+} decreased. (b) In Li^+-salines, tissue water markedly increased with increasing pH; $E_{^{36}Cl^-}$ was of the same order as in Na^+-salines; E_{K^+}, lower at pH 6.2 than in Na^+-saline, significantly decreased; a small electrochemical gradient of Li^+ at pH 6.2 (12 mV) decreased; E_{H^+} was of the same magnitude as in Na^+-salines at all pH.

3. The active transport of α-methyl-D-glucoside at pH 6.2 in the absence of Na^+ was found to be independent of all ionic electrochemical gradients with the exception of Li^+.

Abbreviations: DMO, 5,5-dimethyl-2,4-oxazolidinedione; TES, *N*-tris(hydroxymethyl)-methylaminoethane sulfonate.

It is concluded that the four sugars tested are transported into kidney cortex cells by a variety of pathways which do not appear to be related to the demonstrated active, independent mechanisms for H^+ and K^+ transport. A Li^+ dependency for the active transport of α-methyl-D-glucoside in the absence of Na^+ was observed.

INTRODUCTION

The present investigation was initiated on the basis of a chance observation that the saline pH greatly affected both the rate and near steady-state accumulation ratios of some sugars in kidney cortex cells. A more detailed study of this phenomenon was carried out with the aim of using variations of pH as a tool to elucidate whether one or several pathways are involved in the transport of monosaccharides into renal tubule cells. Indications for a variety of interrelated pathways had previously been obtained on the basis of kinetic data[1]. Also, it was hoped that such studies might contribute to a further understanding of the relationship between Na^+ and the transport of some sugars. Therefore, four sugars were chosen as models for this investigation: 2-deoxy-D-glucose and 2-deoxy-D-galactose, both previously shown to be transported by a Na^+-independent mechanism[2]; and α-methyl-D-glucoside and D-galactose, both of which are transported into kidney cortex cells by essentially Na^+-dependent mechanisms[2–4].

It will be shown here that, with regard to the transport of the above sugars, there are considerable differences in their responses to variations of pH from 6.2 to 8.2. A detailed analysis is also presented below on the relationship between the intracellular ionic concentrations and sugar accumulations with change in pH.

Preliminary data of this investigation have been reported[5,6].

METHODS

As in previous investigations[1], the experiments were carried out using slices from rabbit kidney cortex. The standard experimental procedure was employed, *i.e.* (1) leaching in ice-cold salines for 2.5 h in order to remove tissue components, the effect of which were studied; (2) aerobic (O_2) preincubation in the respective salines at 25° for 45 min and (3) aerobic (O_2) incubation at 25° for 60 min in the presence of investigated sugars or other compounds. Departures from previous described experimental procedures were as follows: variations of saline pH between 6.2 and 8.2 were obtained using 5 ml of an appropriate mixture of 0.308 M Tris and 0.308 M *N*-tris(hydroxymethyl)methylaminoethane sulfonate (TES). The use of this buffer mixture facilitated the maintenance of a stable composition of bulk electrolytes in the media at all pH. pH of the media were determined before and after incubation (initial and final pH) using a pH meter (Radiometer Copenhagen, Model SE26) with a glass electrode.

Na^+- and Li^+-salines were employed. Variations of these salines were prepared as described previously[4].

The distribution of sugars between media and tissue was followed using ^{14}C-

labeled α-methyl-D-glucoside, D-galactose, and 2-deoxy-D-glucose. In addition, nonlabeled 2-deoxy-D-galactose was employed. Unless otherwise stated, the final media concentration of these sugars was 1 mM. The analytical procedures for determining the apparent sugar concentration in the media ($[S]_0$) and tissue $[S]_i$) were identical with those given earlier[1]. In some experiments, the rates of sugar transport were determined (see ref. 1) and the apparent kinetic parameters were calculated. The galactose efflux was followed by the washout technique[3].

In separate experiments, the steady-state tissue water (kg/kg dry wt.) and electrolytes (mequiv/kg dry wt.) were analyzed. After standard leaching, preincubation, and incubation of the tissue with nonlabeled sugar, slices were blotted and the tissue water determined gravimetrically from the difference between tissue wet and dry weight[7]. The dry tissue was then transferred into plastic test tubes and extracted overnight with 5 ml 0.1 M HNO_3 (ref. 8). In the extract, Na^+, K^+ and Li^+ were determined by atomic absorption spectrometry (Evans Electroselenium Ltd., Halstead, Great Britain, Model 140).

The distribution of $^{36}Cl^-$ was obtained as follows: Tissue was incubated under standard conditions in salines labeled with $^{36}Cl^-$ (0.1 μC/ml). Blotted tissue was extracted with 5 ml 0.1 M HNO_3 overnight, and the activity in the extract was determined by scintillation spectrophotometry. Simultaneously, samples of the media before and after incubation were analyzed for $^{36}Cl^-$ after suitable dilution.

The apparent intracellular pH was assayed using the 5,5-dimethyl-2,4-oxazolidinedione (DMO) technique[9,10]. After standard incubation of the tissue in 1 mM [^{14}C]DMO (0.05 μC/ml), the blotted tissue was extracted overnight in 4 ml 0.1 M $NaHCO_3$ and 1 ml of extract used for determination of activity in the scintillation counter. Media samples before and after incubation were similarly analyzed.

The analytical values obtained allowed the calculation of the following data: (1) The near steady-state accumulation ratio for sugars ($[S]_i/[S]_0$), taking into account the values for tissue water independently determined for each experimental condition; from the rates of sugar transport, the apparent kinetic parameters (K_m, v_{max}) and galactose efflux, the rate constants[11]. (2) The apparent ionic concentrations in the cell water and the media (subscripts i and o, respectively), the respective Donnan ratios, and Nernst ionic potentials (E with subscript of the appropriate ionic species); on the basis of previous data[12], $E_{^{36}Cl^-}$ was taken to be identical with the membrane potential. (3) The $[H^+]_i$ calculated from $[[^{14}C]DMO]_i/[[^{14}C]DMO]_0$ at the determined pH_0 (pK for DMO at 25° was determined by potentiometric titration and found to be 6.10).

All data are presented as means ±S.E. where four or more values were obtained. The deviations from the mean analytical values in an individual experiment never exceeded ± 5 %.

Materials

D-[1-^{14}C]Galactose, α-methyl-D-[1-^{14}C]glucoside, 2-deoxy-D-[1-^{14}C]glucose, 5,5-dimethyl-2,4-[2-^{14}C]oxazolidinedione and H^{36}Cl were purchased from New England Nuclear Corp., Boston, and Calbiochem, Los Angeles. 2-Deoxy-D-galactose, TES and 2,4-dinitrophenol were obtained from Sigma Chemical Co., St. Louis, Mo., ethane sulfonic acid from K and K Laboratories, Plainview, N.Y.

RESULTS

Effect of pH on the transport of 2-deoxy-D-glucose

Increasing the pH from approx. 6.2 to 8.2 produced a 3-fold increase in the steady-state accumulation ratios $[S]_i/[S]_0$ both in Na^+- and Li^+-salines (Fig. 1). The $[S]_i/[S]_0$ values were practically identical in both salines at pH 6.2 and 7.2; at pH 8.2 the 2-deoxy-D-glucose accumulation in Li^+-saline was somewhat lower than in Na^+-saline. Previous time-curve experiments established that the transport of 2-deoxy-D-glucose was linear for 30 min and, after 60 min incubation, a steady-state sugar distribution was reached at all pH.

The change of pH from 7.2 to 6.2 doubled the K_m for the transport of 2-deoxy-D-glucose without affecting the v_{max} (see insert on Fig. 1).

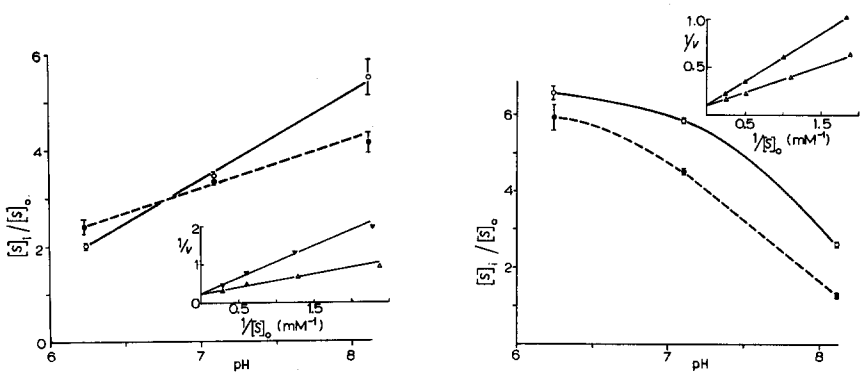


Fig. 1. Effect of pH on the transport of 2-deoxy-D-glucose in kidney cortex cells. After leaching and preincubating, slices were incubated aerobically (O_2) at 25° for 60 min in Na^+-saline (○) or Li^+-saline (●) at the appropriate pH in the presence of 1 mM 2-deoxy-D-glucose. Values ± S.E. (n = 10, two animals). Insert: Lineweaver–Burk plot of the effect of pH on the rate of 2-deoxy-D-glucose transport. Incubation: 30 min. Each experimental point is the mean of three determinations. Apparent kinetic parameters: pH 7.2 (△): K_m 1.4 mM, v_{max} 18 μmoles per g cell water per h; pH 6.2 (▼): K_m 2.8 mM.

Fig. 2. Effect of pH on the transport of 2-deoxy-D-galactose in kidney cortex cells. After leaching and preincubation, slices were incubated aerobically 1 h in Na^+-saline (○) or Li^+-saline (●) at 25° in the presence of 1 mM 2-deoxy-D-galactose. Values ± S.E. (n = 12, two animals). Insert: Lineweaver–Burk plot of the effect of pH on the rate of 2-deoxy-D-galactose transport. Incubation: 30 min. Each experimental point is the mean of three analyses. Apparent kinetic parameters: pH 7.2 (△): K_m 2.5 mM, v_{max} 37 μmoles per g cell water per h; pH 8.2 (▲): K_m 4.8 mM.

Effect of pH on the transport of 2-deoxy-D-galactose

As shown in Fig. 2, varying the saline pH from 6.2 to 8.2 produced an opposite effect on the $[S]_i/[S]_0$ ratio for 2-deoxy-D-galactose transport as compared with 2-deoxy-D-glucose. These changes were comparable in both Na^+- and Li^+-salines. It was noted that the $[S]_i/[S]_0$ ratio for 2-deoxy-D-galactose was slightly lower in Li^+- than in Na^+-saline. The change of pH from 7.2 to 8.2 produced a doubling of the apparent K_m for 2-deoxy-D-galactose transport (see insert to Fig. 2). As for 2-deoxy-D-glucose, previous time-curve experiments have proved the validity for measuring the above kinetic parameters.

Effect of pH on the transport of D-*galactose*

The response of the accumulation of D-galactose in Na^+-saline to change in pH (Fig. 3) was similar to that observed for 2-deoxy-D-glucose, *i.e.* the $[S]_i/[S]_o$ ratio increased nearly 4-fold on increasing the pH from 6.2 to 8.2. In the absence of external Na^+, a slight but definite accumulation of D-galactose against its concentration gradient was observed ($[S]_i/[S]_o$ ratio varied from 1.5 to 2.0); this Na^+-independent fraction of D-galactose accumulation was not markedly affected by pH.

The described effect of pH on the accumulation of D-galactose in Na^+-saline was found to a considerable extent to be reversible (Fig. 4). The experiment was conducted as follows: Slices were incubated for 60 min in a medium of pH 8.2 containing 1 mM D-galactose; after some accumulation of sugar took place, a portion

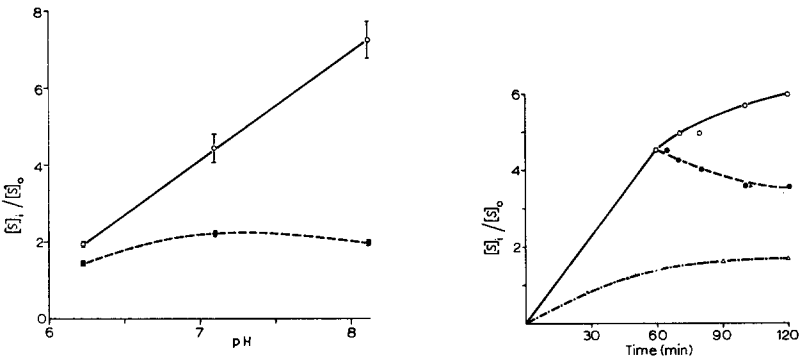


Fig. 3. Effect of pH on the accumulation of D-galactose in kidney cortex cells. After leaching and preincubation, slices were incubated aerobically for 60 min at 25° in either Na^+-saline (○) or Li^+-saline (●) in the presence of 1 mM D-galactose. Values ± S.E. (n = 12, three animals).

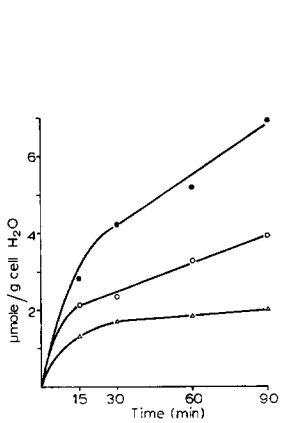
Fig. 4. The reversibility of the pH effect on the transport of D-galactose into kidney cortex cells. Groups of slices were incubated aerobically (O_2) at 25° in Na^+-salines at pH 6.2 (△) and pH 8.2 (○) in the presence of 1 mM D-galactose. After 60 min incubation, a portion of the slices incubated at pH 8.2 was removed and transferred into Na^+-saline at pH 6.2 (●). Groups of slices were removed at varying intervals for analyses. Each point represents the mean of three determinations.

of the tissue was transferred to an identical medium at pH 6.2. Under these conditions a significant loss of tissue D-galactose was observed as compared with some further uptake of sugar by tissue remaining in medium at pH 8.2. For comparison, control values of sugar uptake at pH 6.2 are shown.

An attempt to determine the effect of pH on the kinetic parameters of sugar influx was then made. The results, not reported here, were disappointing in that no interpretable relationship was obtained from a Lineweaver–Burk plot at pH values 6.2 and 8.2, whereas at pH 7.2, a linear relationship was observed as reported previously (refs. 1,3,12). In order to evaluate the reason for the above observations the effect of pH on the time curve of D-galactose transport was therefore studied (Fig. 5). It can be readily seen that the influx of D-galactose can no longer be interpreted as a simple transport system with first-order kinetics. The simplest interpretation of these data is a two-component transport system, both of which are affected by pH. Even at pH 6.2, a close inspection shows that the sugar concentration in

the cells gradually increased as would be expected for such a complex transport system; at higher pH, the second transport component is more evident.

Finally, evidence was obtained that an increase in pH from 7.2 to 8.2 markedly decreased the efflux of D-galactose from the kidney cortex cells (Fig. 6). Particularly, the slow efflux component was affected (k''_2 at pH 7.2 = 0.012 min^{-1}; k''_2 at pH 8.2 = 0.008 min^{-1}).



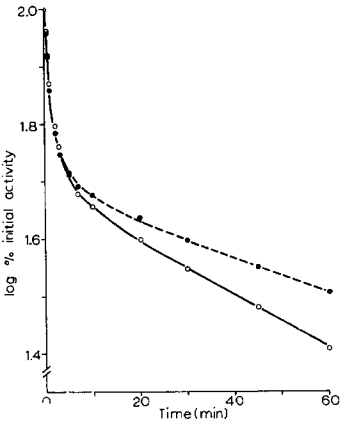


Fig. 5. Time-curve of D-galactose transport into kidney cortex cells at varying pH. Slices were preincubated aerobically (O_2) at 25° in Na^+-salines of the appropriate pH for 45 min, then transferred into identical salines containing 1 mM D-galactose and incubated aerobically for different time intervals. Each value represents the mean of three analyses. Saline pH: 6.2 (△), 7.2 (○) and 8.2 (●).

Fig. 6. Effect of pH on the efflux of galactose from kidney cortex slices. Slices were first loaded with sugar by aerobic incubation for 90 min at 25° in saline (pH 7.2) containing 1 mM D-[^{14}C]-galactose (0.2 µC/ml). The blotted slices ($[S]_1/[S]_0 = 2.52$) were placed into wash-out tubes and the efflux of the sugar into two series of tubes, each containing 10 ml sugar-free salines of pH 7.2 (○) and 8.2 (●) was followed. Ordinate: log % of initial tissue activity.

Effect of pH on the transport of α-methyl-D-glucoside

Fig. 7 shows that in Na^+-saline variations of pH do not greatly affect the accumulation of α-methyl-D-glucoside under given experimental conditions. Surprisingly,

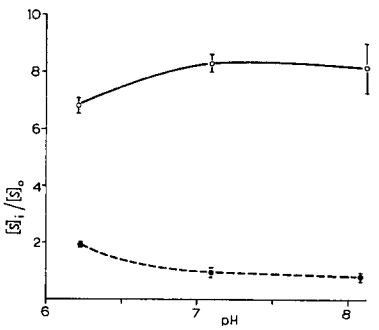


Fig. 7. Effect of pH on the accumulation of α-methyl-D-glucoside in kidney cortex cells. After leaching and preincubation slices were incubated aerobically for 60 min at 25° in either Na^+-saline (○) or Li^+-saline (●) in the presence of 1 mM α-methyl-D-glucoside. Values ± S.E. (n = 12, three animals).

a small but highly significant active accumulation of α-methyl-D-glucoside was observed in the absence of external Na^+ at pH 6.2 (mean $[S]_i/[S]_0$ ratio = 1.725 ± 0.101 S.E.; ten animals); at higher pH, the $[S]_i/[S]_0$ ratios were below 1.0 indicating a lack of active transport. All previous experiments with this sugar at pH 7.4 indicated an absolute requirement of external Na^+ for its accumulation against a concentration gradient[2,4].

The following data provided evidence that the transport of α-methyl-D-glucoside at pH 6.2 at $[Na^+]_0 = 0$ (and $[Na^+]_i < 3$mM) is active (Table I). The accumulation of α-methyl-D-glucoside was (a) ouabain insensitive, as would be expected for Na^+-independent transport; (b) inhibited by 0.1 mM 2,4-dinitrophenol and anaerobiosis to $[S]_i/[S]_0$ values below one, demonstrating the metabolic dependence of the transport system; (c) inhibited by 0.1 and 0.5 mM phlorrhizin, implying the participation of a carrier-mediated system; and (d) only minimally affected by 0.1 and 0.5 mM acetazolamide, indicating that carbonic anhydrase does not significantly participate in the establishment of conditions favorable to the active transport of this sugar.

TABLE I

EFFECT OF VARIOUS EXPERIMENTAL CONDITIONS ON THE ACCUMULATION OF α-METHYL-D-GLUCOSIDE AT pH 6.2 IN Li^+-SALINE

Slices were first leached and preincubated in Li^+-saline at pH 6.2, then incubated 60 min at 25° in salines (Li^+, pH 6.2) containing 1 mM α-methyl-D-[^{14}C]glucoside or, in a separate experiment, 1 mM [^{14}C]DMO, and various additions. Values of $[S]_i/[S]_0$ are means ± S.E. where four or more determinations were carried out; values of $[DMO]_i/[DMO]_0$ are the means of three determinations.

Expt. No.	*Additions*	*Concn. (mM)*	$[S]_i/[S]_0$ *ratio*	$[DMO]_i/[DMO]_0$ *ratio*
1	Control		1.75 ± 0.03	2.28
	Ouabain	0.1	1.65 ± 0.01	
	Ouabain	0.5	1.69 ± 0.01	
	2,4-Dinitrophenol	0.1	0.75 ± 0.01	1.45
	Acetazolamide	0.1	1.58 ± 0.01	
	Acetazolamide	0.5	1.53 ± 0.02	2.36
2	Control		1.60 ± 0.04	
	Anaerobiosis (He)		0.78 ± 0.01	
3	Control		2.09	
	Phlorrhizin	0.1	0.78	
	Phlorrhizin	0.5	0.55	

Effect of pH on the ionic distribution in kidney cortex cells

The data presented above showed marked differences in the response of the transport systems of the four model sugars to changes in pH. These observations raised the question whether a relationship could be established between cellular ionic distribution and the transport of the above sugars. Accordingly, the effect of pH on the steady-state levels of water, Na^+, K^+, Li^+ and H^+ were studied. In addition, the distribution of $^{36}Cl^-$ was determined as a measure of membrane potential. From the obtained data, the apparent intracellular ionic concentrations were calculated. Tables II and III summarize the results.

In Na^+-saline (Table II), variations of pH between 6.2 and 8.2 had little effect

TABLE II

EFFECT OF pH ON THE STEADY-STATE ION AND WATER DISTRIBUTION IN KIDNEY CORTEX SLICES INCUBATED IN Na^+-SALINES

Slices were aerobically (O_2) preincubated (45 min) and incubated (60 min) at 25° in Na^+-salines of varying pH. Portions of the salines were labeled with $^{36}Cl^-$ (0.1 μC/ml, $[Cl^-]_0$ = 135 mM) or 1 mM [^{14}C]DMO (0.05 μC/ml). All values are the means ± S.E. of at least ten analyses (two animals).

Final pH	*6.30*	*7.14*	*8.11*
Tissue contents			
Water (kg/kg dry wt.)	3.03 ± 0.05	2.85 ± 0.05	3.15 ± 0.06
Na^+ (mequiv/kg dry wt.)	222 ± 11	236 ± 17	333 ± 22
K^+ (mequiv/kg dry wt.)	364 ± 8	315 ± 11	260 ± 13
Li^+ (mequiv/kg dry wt.)	26 ± 3	25 ± 3	40 ± 2
Apparent intracellular concn. (mM)			
$[Na^+]_i$	46 ± 7	60 ± 7	95 ± 18
$[K^+]_i$	177 ± 7	165 ± 7	120 ± 8
$[Li^+]_i$	10 ± 2	10 ± 2	16 ± 2
$[^{36}Cl^-]_i$	67 ± 3	52 ± 2	70 ± 4
$[H^+]_i$	11.5 ± $0.3 \cdot 10^{-5}$	3.1 ± $0.2 \cdot 10^{-5}$	0.81 ± $0.02 \cdot 10^{-5}$

TABLE III

EFFECT OF pH ON THE STEADY-STATE ION AND WATER DISTRIBUTION IN KIDNEY CORTEX SLICES INCUBATED IN Li^+-SALINES

Slices were first leached 2.5 h in Li^+-saline, then aerobically (O_2) preincubated (45 min) and incubated (60 min) in Li^+-salines of varying pH. Portions of salines were labeled with $^{36}Cl^-$ (0.1 μC/ml, $[Cl^-]_0$ = 135 mM) or 1 mM [^{14}C]DMO (0.05 μC/ml). All values are the means ± S.E. of at least ten analyses (two animals).

Final pH	*6.24*	*7.11*	*8.11*
Tissue contents			
Water (kg/kg dry wt.)	2.73 ± 0.02	3.11 ± 0.04	3.97 ± 0.07
Na^+ (mequiv/kg dry wt.)	5 ± 3	3 ± 0	3 ± 0
K^+ (mequiv/kg dry wt.)	160 ± 5	85 ± 1	77 ± 1
Li^+ (mequiv/kg dry wt.)	376 ± 6	517 ± 20	666 ± 16
Apparent intracellular concn. (mM)			
$[Na^+]_i$	3 ± 2	1 ± 0	1 ± 0
$[K^+]_i$	86 ± 3	37 ± 1	25 ± 1
$[Li^+]_i$	139 ± 5	181 ± 11	183 ± 11
$[^{36}Cl^-]_i$	66 ± 1	84 ± 2	113 ± 2
$[H^+]_i$	16 ± $0.8 \cdot 10^{-5}$	4.2 ± $0.3 \cdot 10^{-5}$	0.81 ± $0.02 \cdot 10^{-5}$

on the tissue water content but had significant effects on the tissue content of Na^+ and Li^+ (increase with increasing pH) and on K^+ (decrease with increasing pH). Changes in similar directions were noted for the respective intracellular concentrations of these ions. An analysis of the $[^{36}Cl^-]_i$ showed only small effects of pH, while the [^{14}C]DMO distribution indicated a decrease of $[H^+]_i$ with increase in pH.

Table III illustrates the effect of pH on the ionic distribution in Li^+-saline. The following points are of particular interest: The steady-state level of tissue water

markedly increased with increasing pH; since this change occurred in the absence of $[Na^+]_0$ (and at very low $[Na^+]_i$), the major actively transported ion, the "leak and pump" hypothesis (see ref. 14) hardly can explain the volume regulation of the cells, particularly at lower pH. The K^+ tissue content markedly decreased while tissue Li^+ increased with increasing pH; their respective intracellular ion concentrations varied in similar directions. The $[^{36}Cl^-]_i$ increased and $[H^+]_i$ decreased with elevation in pH.

It should be stated that during the incubation of the tissue, small but systematic changes of pH in both Na^+- and Li^+-salines occurred: at pH 6.2 a mean alkalinization of 0.21 pH units of the media took place corresponding to 4 μequiv of OH^-; at pH 7.2 an acidification of the media occurred but usually less than 0.05 pH units (3 μequiv of H^+); at pH 8.2 an acidification was observed of 0.09 pH units (4 μequiv of H^+).

From the data in Tables II and III, the apparent electrochemical potentials of the individual ionic species were evaluated by plotting the apparent Nernst diffusion potentials against pH (Figs. 8 and 9). Since $E_{^{36}Cl^-}$ can be taken as a measure of membrane potential, the steady-state electrochemical potential for a given ion is then determined by the difference: E_{ion} *minus* $E_{^{36}Cl^-}$.

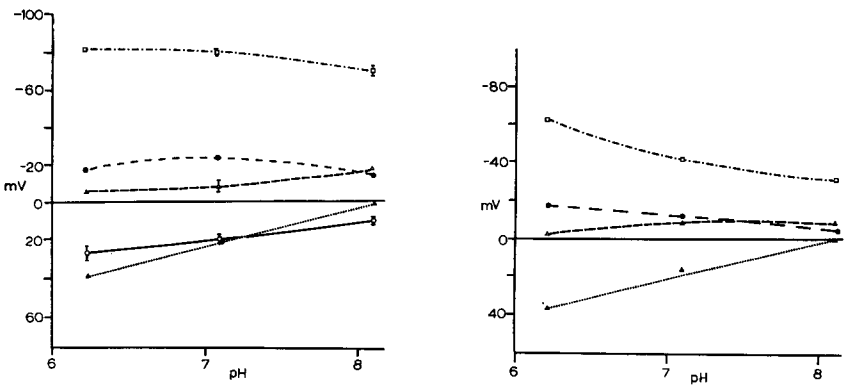


Fig. 8. Effect of pH on the steady-state Nernst ionic potentials in kidney cortex cells incubated in Na^+-salines. For experimental conditions, see Table I. ●, $E_{^{36}Cl^-}$; △, E_{Li^+}; □, E_{K^+}; ○, E_{Na^+}; ▲, E_{H^+}. Means ± S.E. (n = 8, two animals).

Fig. 9. Effect of pH on the steady-state Nernst ionic potentials in kidney cortex cells incubated in Li^+-salines. For experimental conditions, see Table II. ●, $E_{^{36}Cl^-}$; △, E_{Li^+}; □, E_{K^+}; ▲, E_{H^+}. Means ± S.E. are given (n = 8, two animals).

From Fig. 8, it follows that both E_{Na^+} and E_{H^+} are opposite in sign to $E_{^{36}Cl^-}$; thus, an extrusion of both these ions takes place against their electrochemical gradients. This appears to be the first instance where the active secretion of H^+ was directly demonstrated *in vitro* in kidney cortex. STRUYVENBERG *et al.*[15] were able to find only indirect evidence for active H^+ transport under their experimental conditions in canine renal tubule cells. It should be noted that in the presence of 2,4-dinitrophenol (Table I), the $[DMO]_i/[DMO]_0$ ratio significantly decreased, indicating that the ability of kidney cortex cells to maintain a H^+ gradient by actively secreting H^+ is metabolically controlled. A small but significant electrochemical

gradient for Li^+ in the direction of intracellular to extracellular components was observed at pH 6.2 and 7.2 but abolished at 8.2. The electrochemical gradient of K^+ was not markedly affected by pH changes.

Fig. 9 illustrates a similar analysis of the electrochemical potentials of ions distributed in tissues incubated in Li^+-saline. The $^{36}Cl^-$ potential was not greatly affected by pH and was of a similar magnitude as that found in Na^+- saline(Fig. 8). Again, a marked H^+ extrusion was observed, E_{H^+} varying with pH in the same way as in Na^+- saline. A small electrochemical gradient due to the extrusion of Li^+ was noted at pH 6.2. Na^+ potentials were so small that they could reasonably be neglected. There was a significant decrease of E_{K^+} with increasing pH. This is indicative of an active K^+ transport independent of Na^+ since the electrochemical potential of K^+ was established in the absence of Na^+_0. Such a "K^+ pump" has been postulated for kidney cortex cells earlier[12] and has been demonstrated *in vivo* in the proximal kidney tubule[16].

The relationship between ionic distribution and sugar transport

The results reported in the previous sections do not provide any direct information as to the observed effects of pH and ionic distribution on the transport of the four model sugars. For instance, the effect of pH on the Na^+-independent transport of 2-deoxy-D-glucose (Fig. 1) was opposite to that on 2-deoxy-D-galactose (Fig. 2). Furthermore, it has been demonstrated previously[4] that the transport of both α-methyl-D-glucoside and D-galactose are stimulated by saline K^+. However, in the absence of Na^+ in the media (where the electrochemical potential of K^+ decreases), an increase of pH from 7.2 to 8.2 produced an increase in the accumulation of D-galactose (Fig. 3) and a decrease in the accumulation of α-methyl-D-glucoside (Fig. 7).

An effort was therefore made to analyze further the Na^+-independent transport of α-methyl-D-glucoside at pH 6.2 attempting to relate this transport to the ionic distribution.

The ionic requirement for α-methyl-D-glucoside transport in the absence of Na^+

The specificity of the ion requirement of α-methyl-D-glucose transport was first examined by employing modified salines (Table IV). The data show that the active accumulation of α-methyl-D-glucoside was not affected by the absence of Ca^{2+} or Cl^- and somewhat reduced by an increase in the K^+ of the saline. Table V presents the results on the water content and the sugar and ionic distributions in Li^+-saline in the presence and absence of K^+. With the omission of K^+_0, the $[K^+]_i$ was reduced to nearly one-half of that in the control while the sugar accumulation and the H^+_i and $^{36}Cl^-$ distribution remained unchanged. It thus may be concluded that Ca^{2+}, Cl^-, or K^+ are not related to the transport of this sugar. On the other hand, the absence of Li^+ (choline$^+$- and Tris$^+$-salines) abolished the active accumulation of α-methyl-D-glucoside reducing the $[S]_i/[S]_0$ ratio to values below one. This result suggested a Li^+ dependence of the observed sugar transport in the presence of the small electrochemical gradient of this ion (12 mV); however, the possibility that the H^+ gradient is responsible for the α-methyl-D-glucoside transport had also to be considered. Therefore, the $[DMO]_i/[DMO]_0$ ratios were determined for a variety of experimental conditions (Tables I, IV, and V). It will be noted from Table IV that

TABLE IV

EFFECT OF THE SALINE ELECTROLYTE COMPOSITION ON THE ACCUMULATION OF α-METHYL-D-GLUCOSIDE AT pH 6.2

Slices were first leached and preincubated in the respective salines, at pH 6.2, then incubated aerobically (O_2) at 25° for 60 min in the respective salines with 1 mM α-methyl-D-[^{14}C]glucoside or, in a separate experiment, with [^{14}C]DMO. Values of $[S]_i/[S]_0$ are the means of three determinations. See METHODS for description of salines.

Saline	$[S]_i/[S]_0$ *ratio*	$[DMO]_i/[DMO]_0$ *ratio*
Li^+-saline		
Control	1.55 ± 0.04	2.27
Ca^{2+}-free	1.51 ± 0.06	2.46
25 mM K^+	1.25 ± 0.04	
Cl^--free	1.66 ± 0.05	1.93
Choline$^+$-saline	0.95 ± 0.04	2.29
Tris$^+$-saline	0.94 ± 0.01	2.32

TABLE V

EFFECT OF K^+ ON THE STEADY-STATE ACCUMULATION OF α-METHYL-D-GLUCOSIDE AND DISTRIBUTION OF IONS IN Li^+-SALINE AT pH 6.2

Groups of slices were first leached in ice-cold K^+-free saline for 2.5 h, then aerobically (O_2) preincubated and subsequently incubated 60 min at 25° standard saline (6.7 mM K^+, control) and in K^+-free saline, both containing 1 mM α-methyl-D-glucoside. Separate groups of slices were incubated in salines labeled with $^{36}Cl^-$ (0.1 μC/ml) or [^{14}C]DMO (0.05 μC/ml). Tissue contents of water and ions are given as well as the computed apparent intracellular ionic concentrations and the Donnan and sugar accumulation ratios. All values are the means ± S.E. (n = 10, two animals).

K^+ (*mM*)	6.7	0
Sugar $[S]_i/[S]_0$	1.41 ± 0.04	1.59 ± 0.13
Tissue contents		
H_2O (kg/kg dry wt.)	2.83 ± 0.05	2.72 ± 0.03
Na^+ (mequiv/kg dry wt.)	2.2 ± 0.3	2.7 ± 0.4
K^+ (mequiv/kg dry wt.)	117 ± 3	62 ± 2
Li^+ (mequiv/kg dry wt.)	438 ± 15	507 ± 25
Apparent intracellular concn. (*mM*)		
$[Na^+]_i$	1.2 ± 0.2	1.5 ± 0.3
$[K^+]_i$	61 ± 2	35 ± 2
$[Li^+]_i$	186 ± 13	234 ± 17
$[H^+]_i$	19 ± $0.4 \cdot 10^{-5}$	19 ± $0.1 \cdot 10^{-5}$
Distribution ratios		
$[K^+]_i/[K^+]_0$	9.09	—
$[Li^+]_i/[Li]_0$	1.38	1.73
$[^{36}Cl^-]_0/[^{36}Cl]_i$	2.03	1.98
$[DMO_i]/[DMO]_0$	2.05	2.05
$[H^+]_i/[H^+]_0$	0.36	0.35

the accumulation of α-methyl-D-glucoside was abolished by choline$^+$- and Tris$^+$-salines while the $[DMO]_i/[DMO]_0$ ratios were identical with the control. On the other hand, the $[DMO]_i/[DMO]_0$ ratio was significantly decreased in Cl^--free saline while the $[S]_i/[S]_0$ ratio was unchanged. One may therefore conclude that the sugar accu-

mulation is not markedly related to H^+ distribution, and a Li^+ dependence of the transport of α-methyl-D-glucoside at pH 6.2 in the absence of Na^+ may be indicated.

The inhibition pattern of α-methyl-D-glucoside accumulation at pH 6.2 by competing sugars was found to be similar in both Li^+- and Na^+-salines: D-glucose and D-galactose markedly inhibited the $[S]_i/[S]_0$ ratio whereas 2-deoxy-D-glucose was not greatly effective (Table VI). It may thus be assumed that the same pathway is used for the α-methyl-D-glucoside transport in both salines. However, the alternative possibility, separate Li^+-dependent and Na^+-dependent transport pathways, cannot at present be excluded.

TABLE VI

COMPETITION OF VARIOUS SUGARS FOR THE ACCUMULATION OF α-METHYL-D-GLUCOSIDE IN KIDNEY CORTEX CELLS AT pH 6.2

Slices were first leached for 2.5 h in ice-cold Li^+-saline, then aerobically (O_2) preincubated 45 min in either Li^+- or Na^+-salines (pH 6.2) and incubated 60 min in these salines containing 1 mM α-methyl-D-glucoside without (control) and with other sugars. All values are the means of three analyses.

Sugar added	*Concn. (mM)*	*Li^+-saline $[S]_i/[S]_0$ ratio*	*Na^+-saline $[S]_i/[S_0$ ratio*
Control		1.62	7.67
D-Glucose	5	1.35	2.59
D-Galactose	5	1.39	4.33
2-Deoxy-D-glucose	5	1.69	6.52

DISCUSSION

From previous evidence[2,3] the sugar transport by kidney cortex cells could be classified as follows: (1) sugars showing an absolute requirement of external Na^+ for their active transport, *e.g.* α- and β-D-glucosides, D-galactose, *etc.* and (2), 2-deoxyhexoses which are transported by a Na^+-independent mechanism. In view of a mutual competitive inhibition of sugar transport within each group as well as between both groups, the possibility of a common transport pathway was envisaged[2]. In the case of the 2-deoxyhexoses, the found kinetic parameters appeared to be consonant with such an assumption[1].

Data shown in Fig. 1,2,3 and 7 now lend emphasis to the revised view[1,4] that a variety of transport mechanisms participate in the accumulation of the four model sugars tested here: an increase in Na^+-saline pH from 6.2 to 8.2 produced an increase in both the rate and near steady-state accumulation of 2-deoxy-D-glucose and D-galactose, no major effect on the transport of α-methyl-D-glucoside, and a decrease in both the rate and near steady-state accumulation of 2-deoxy-D-galactose. Such variations in the response to pH changes of sugar transport cannot be due to a general effect on metabolic processes. The above data therefore indicate essential differences between the transport systems of 2-deoxy-D-glucose and 2-deoxy-D-galactose in spite of their competitive transport inhibition[1]. Also, some differences between the transport of D-galactose and α-methyl-D-glucoside have to be considered in view of their varied responses to pH changes; such a possibility has been indicated from kinetic data where the K_i for inhibition was nearly one order of magnitude

higher than the transport K_m of the respective sugar[1]. Finally, a comparison of the Na^+-independent transport of 2-deoxy-D-glucose and D-galactose (Figs. 1 and 3) suggests that at least a portion of the D-galactose transport cannot utilize a common pathway with 2-deoxy-D-glucose. We are thus faced with evidence for a multiplicity of transport pathways (and/or mechanisms) for sugars with overlapping specificities indicated by kinetic data for competitive inhibitions[1]. Since any active transport is assumed to involve an interaction between the transported solute and a carrier (the nature of which is probably a protein), the marked effects of pH on the sugar transport within the range 6.2–8.2 may be indicative of a system (carrier) with a pK in this range; one such possibility would be a role for the amino group of histidine (pK = 8.00).

It would be premature at present to speculate about the localization of these pathways and/or mechanisms at the polar faces of the kidney tubular cells; in this context, evidence obtained *in vivo*[17] showed that D-galactose can enter the cells of canine proximal tubule by two mechanisms localized at the tubular face of the cells as well as a system localized at the peritubular face. Several pathways (and/or mechanisms) of D-galactose transport may therefore have been responsible for the complex entry kinetics of the sugar (Fig. 6). The kinetic parameters given previously[1] for D-galactose transport at pH 7.4 appear to be composite values for at least two transport processes. If these two transport phenomena were differently and independently affected by change in pH, Lineweaver–Burk plots for D-galactose would yield data not readily interpretable unless exceedingly short time intervals were chosen for the determination of the kinetic parameters. Unfortunately, such a procedure is not feasible because the size of the extracellular compartment would then limit the calculation of intracellular sugar concentration.

The second point of interest concerns the Na^+ dependence of the transport of the four model sugars and the effect of pH on this phenomenon. Figs. 1 and 2 demonstrate once again the Na^+ independence of the transport of both 2-deoxyhexoses tested (*cf.* refs. 2 and 4); thus kidney cells differ from the intestinal cells where an absence of an active transport of 2-deoxy sugars has been repeatedly demonstrated[18,19] and no Na^+-independent transport of any sugar has been observed.

With regard to the transport of sugars previously considered to take place by a Na^+-dependent mechanism, the data shown in Fig. 3 confirm indications[4] that a portion of the D-galactose transport proceeds by a Na^+-independent mechanism. Furthermore, a Na^+-independent active transport of α-methyl-D-glucoside has now been demonstrated at pH 6.2 (Fig. 7). The question thus has to be raised whether any sugar transport in kidney cortex cells will proceed only in the presence of Na^+. In this context, two reports should be mentioned. CSÁKY AND RIGOR[20] have demonstrated that a considerable portion of sugars can be actively transported into the epithelial cells of the chorioid plexus by a Na^+-independent mechanism. Recently, it was reported[21] that even in the intestinal mucosa, a dissociation of the transport of a nonelectrolyte (L-alanine) and Na^+ has been achieved by lowering the pH of the medium. The relevance of the observed differences of the Na^+ requirement for the sugar transport in kidney cortex cells to the current hypothesis of the mechanism of coupling between Na^+ and nonelectrolyte transport has been previously discussed[4].

In order to evaluate the possible driving forces for the Na^+-independent

active transport of α-methyl-D-glucoside at pH 6.2, the effect of pH on the ionic distribution has to be first considered.

Attention should be given to four salient observations arising out of the data presented in Tables II, III and V and Figs. 8 and 9:

(1) A H^+ transport against its electrochemical gradient in the direction of cell to media was clearly demonstrated. Such active extrusion of H^+ has been postulated on the basis of experiments *in vivo* in kidney[22]. The existence of an active H^+ secretion does not necessarily lead to an acidification of the media; the oxidation of organic substrates and the pK of carbonic acid thus formed has to be considered, as well as the system employed *in vitro*. The extrusion of H^+ at one pole of gastric mucosa cells is known to be associated with the appearance of an equivalent amount of HCO_3^- at the other cellular pole[23,24]. Using kidney cortex slices, this mechanism would lead to $H^+ + HCO_3^- \rightleftharpoons H_2O + CO_2$ with only a negligible change in pH. On the other hand, the oxidation of the metabolic substrate used, acetate, would lead to the net formation of $Li^+ + HCO_3^-$ and, consequently, to a possible alkalinization of the media; such alkalinization in fact was observed at pH 6.2 in both Na^+- and Li^+-salines. Slight acidification of the media at pH 7.2 and 8.2 could be accounted for by the increased dissociation of H_2CO_3.

The demonstrated H^+ pump does not appear to be linked to K^+ transport (*i.e.* a H^+–K^+ exchange pump)[22], since the absence of external K^+ did not affect the electrochemical gradient of H^+ (Table V). Also, no link to Na^+ transport was apparent, E_{H^+} not being affected by the absence of Na^+_0 (Fig. 9).

(2) The fact that the electrochemical potential of K^+ was only slightly lowered in the absence of Na^+ (*cf.* Figs. 8 and 9) indicated an accumulation of K^+ against its electrochemical gradient independent of the Na^+ pump. This point is apparent from an inspection of Table V which shows that in the absence of Na^+ at pH 6.2, the steady-state $[K^+]_i$ was 26 mequiv/kg water higher in the control ($[K^+]_0 = 6.7$ mM) than in tissues incubated in K^+-free saline; at $[K^+]_0 = 0$, the value for $[K^+]_i$ may well represent the amount of cellular K^+ that does not appear to participate in osmotic and electrochemical phenomena in kidney cortex cells[12].

(3) The marked effect of pH on the E_{Na^+} should be noted indicating a decrease in the activity of the Na^+ pump parallel to the decrease of H^+ concentration (Fig. 8). In this regard, it will be recalled that a decrease of the Na^+ short-circuit current in the frog skin by increasing the pH has been observed by USSING[25]. While the Na^+ extrusion could readily explain the observed $E_{^{36}Cl^-}$ in Na^+-saline (Fig. 8), no explanation is available for the mechanism by which the unequal distribution of $^{36}Cl^-$ is brought about in the absence of Na^+.

(4) It was observed that the values of tissue water (which can be taken as a measure of cell volume) did not markedly change in Na^+-saline with variations in pH. On the other hand, in Li^+-saline the cells were capable of maintaining their volume at the same level as in Na^+-saline at pH 6.2 and 7.2, but a further increase of pH produced a significant swelling of the cells. This control of cell volume in the absence of Na^+ at pH 6.2 was found to be metabolically dependent; the presence of 0.1 mM 2,4-dinitrophenol (results not reported here) produced a marked swelling of the cells. These observations are difficult to reconcile with the "leak and pump" hypothesis as the only mechanism for the regulation of cell volume (see ref. 14). An analysis of this phenomenon will be presented elsewhere.

Finally, it should be noted that there was no clear-cut correlation between the effect of pH on ionic distribution and sugar transport. With regard to the Na^+-independent active transport of α-methyl-D-glucoside, which was found to take place only at pH lower than 7.0, experiments presented in Tables I, IV and V appear to exclude its coupling to the transport of all media components except Li^+. The results of competitive-inhibition studies (Table VI) suggest that the transport pathway for α-methyl-D-glucoside may be the same in both Na^+- and Li^+-salines. However, the possibility of separate pathways cannot so far be excluded. The small electrochemical gradient of Li^+ at pH 6.2 in the absence of Na^+ may provide a flux sufficient for the transport of this sugar. Alternatively, the presence of a mobile cation (such as Na^+ or Li^+) near the carrier in the membrane may be the only cation requirement for sugar transport. In any case, the reported observation of an apparent Li^+ dependence of a sugar transport previously considered to be entirely Na^+ dependent questions the specificity of the postulated mandatory Na^+ requirement for such transport. It is of interest to note that Li^+, as opposed to choline, was found to faciltitate sugar equilibration between a Na^+-free medium and intestinal epithelial cells[26].

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